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Closed-circuit organ perfusion technique for gene transfer into the lungs. An experimental trial on farm pigs

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Abstract

Background In an attempt to develop gene therapy for lung diseases, we have explored a closed-circuit surgical perfusion method for gene transfer into the lung.

Materials and methods For gene transfer we used a replication defective type 5 adenovirus carrying the *E. coli* β-galactosidase gene as a reporter gene. The middle lobe of the right lung of eight young farm pigs was perfused *in vivo* via thoracotomy for up to 60 min with the viral solution. The gene transfer was performed using a closed-circuit organ perfusion method *in vivo*. The efficiency of gene transfer was assessed visually by analysis of histologic sections after X-gal, PAS and immunohistochemical stainings.

Results The lung perfusion resulted in transgene expression in the alveolar epithelial cells, capillary endothelial cells, airway epithelial cells and alveolar macrophages of the lung examined seven days after perfusion.

Conclusion The present results suggest that operatively performed closed-circuit warm lung perfusion method may be used for gene transfer in treatment of diseases that have pulmonary manifestations.

Keywords gene therapy, gene transfer, inherited diseases, lung perfusion
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Introduction

Gene therapy, the introduction of therapeutic genetic material into affected cells or organs to correct a disease phenotype, is evolving into a method of treatment for human illness. Advances in recombinant-DNA technology have facilitated the introduction of therapeutic genes into somatic cells. In recent years, several clinical trials involving human gene therapy have been accepted by regulatory agencies for a large variety of diseases [1,2]. Disorders, such as the most common inherited lung disorders cystic fibrosis (CF) [3,4], as well as α1-antitrypsin deficiency [5,6] are candidates for somatic lung gene therapy. The first reports of *in vitro* correction of the CF chloride channel defect were published in 1990 [3] and *in vivo* CF gene expression was established in the airways of mice in 1992 [4]. Gene therapy is also being considered for the treatment of acquired inflammatory and infectious diseases of the lung [7], lung cancer [8], the surfactant protein B deficiency [9] and in conjunction with lung transplantation for preventing reperfusion injury and transplant rejection [10].

The chloride channel defect of CF primarily affects lung epithelia, although other tissues such as the pancreas are usually also affected. Attempts to correct the CF lung complications have mainly focused on gene transfer to the epithelial cells of the airways and alveoli. Thus, expression of foreign genes in epithelial cells of the airways has been achieved using virus or liposome containing aerosols [11,12] or via intratracheal instillations of vector containing solutions through a bronchoscope or tracheostomy [13,14]. However, the transgene expression in the alveolar cells has been mainly observed in macrophages, while the actual target cells for CF gene therapy, the epithelial cells have shown very limited expression of the transgene.

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Replication defective adenovirus has been extensively and successfully used for transfer of genes into pulmonary cells in culture and *in vivo* [15,16]. Adenoviruses have many advantages as gene transfer vectors. Their safety has been proved in use for human vaccination for years. Although adenoviruses are common human pathogens, they have not been associated with human neoplasms. They can be grown in high titers and carry large pieces of exogenous DNA. They are naturally tropic for the respiratory epithelium and they can infect nondividing cells. However, previous studies have demonstrated that adenoviral transfection is only transient and yields overall inefficient gene expression [17].

Another means for achieving gene transfer into lung is via the vascular route. Thus far, intravascular infusions have yielded quite inefficient gene transduction [18,19]. *Ex vivo* gene transfer into explanted cultured cells and implantation of the transfected cells has been used for gene transfer into bronchial epithelial cells in an animal model [20]. Also, direct injection into lung tumours has been used [21]. The drawbacks of these methods are that the transduction is not selective and that significant amounts of the therapeutic gene-containing vector may be needed.

Extensive research is being carried out to enhance the efficacy of gene transfer into the target cells *in vivo*. Thus, gene transfer efficiency has been reported to improve by pretreatment with host barrier properties modificating agents, e.g. polidocanol before vector administration [22]. Modification of the hosts immune system may enhance the transgene expression and enable repeated administrations concerning viral mediated gene transfer [23]. Another approach reported to enhance gene transfer efficacy is to prolong the incubation time with the vector and the target cells in different tissues [24,25]. In order to increase the interaction time between a vector and target cells, we developed a surgical closed-circuit organ perfusion method that resulted in efficient gene transfer into the cells of up to 85% of renal glomeruli. In the present study, we have applied this organ perfusion method to target adenovirus-mediated gene transfer into lung cells of farm pigs. Following 60 min warm lung perfusion, the expression of the *lacZ* reporter gene could be observed in airway and alveolar cells, including epithelial cells.

Materials and methods

Reporter gene virus

A replication defective recombinant first-generation serotype 5 adenovirus (AdCMV*lacZ*) containing the cytomegalovirus promoter and the *E. coli* β-galactosidase gene as a reporter gene was used as a gene transfer vector [26]. Sequences in the El A, El B and E3 regions have been deleted. Adenovirus stocks were prepared and viral titers were determined by plaque assay and expressed as plaque forming units per millilitre (pfu mL^{-1}) as previously described [26]. Expression of the reporter gene serves to identify cells where successful transduction has occurred.

Experimental animals

Experimental animals were young 22–35 kg farm pigs, which received human care in compliance with the guidelines established by the National Institutes of Health and the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 86–23, revised 1985). First, one *ex vivo* lung perfusion was made at 37 °C for 10 hours to give time for the possible transgene expression to become visible. In this study, operative *in vivo* gene transfer trial was made for 11 animals. The animals were under general anesthesia during the operation. Azaperon (Stresnil; Janssen Pharmaceutica, Beerse, Belgium) 4 mg kg^{-1} was first administered as intramuscular injection. For induction medetomidine (Domitor; Lääkefarmos, Orion, Espoo, Finland) 80 μg kg^{-1}, ketamin (Ketalar; Parke-Davis, Täby, Sweden) 4 mg kg^{-1} and atropine (Atropin; Leiras, Turku, Finland) 0·05 mg kg^{-1} were given intramuscularly. Thiopenthal (Pentothal Natrium; Abbott, Illinois, USA) 5 mg kg^{-1} was then given intravenously, the animal was intubated into the left main bronchus and the anesthesia was continued under a combination of nitrous oxide–oxygen (1:1) and 1·5% enflurane (Efrane; Abbott, Italy).

Lung perfusion system

A closed-circuit perfusion system designed for continuous organ circulation *in vivo* [27] was used. The system consisted of a reservoir for the perfusate, a roller pump, an artificial membrane lung, and the lung lobe to be perfused, all connected by silicon tubing with 3 mm inside diameter (OT-Silicon, Ii, Finland) (Fig. 1). The *in vivo* perfusions were carried out into the middle lobe of the right lung.

A right intercostal thoracotomy incision was made. After dissection of the hilum the vascular clamps were placed proximally in the right middle lobe pulmonary artery and vein distally at the hilum. The lung was attached to the perfusion system by cannulating the artery of middle lobe with a 18G PTFE cannula (Venflon, Helsingborg, Sweden) and the vein with a 16G PTFE cannula (Venflon). The warm ischemia lasted 10 minutes during the cannulation in all experiments. The venous effluent was collected directly to the reservoir. The perfusate had a total volume of 350 mL and contained previously-separated porcine red blood cells at a haematocrit value of 0·17 in Krebbs-Ringer solution. Additionally, heparin 25 000 IU (Heparin Leo, Lövens, Ballerup, Denmark) and kefuroxim (Lifurox, Eli Lilly, Indianapolis, Indiana Y6285, USA) 250 mg as antibiotic were added into the perfusate. The pH and oxygen saturation in the perfusate were measured by laboratory blood gas analysis from the perfusate during the perfusion operation.

Before connecting the lung to the perfusion system, a 10 mL lidocain-heparin solution (190 mg lidocain (Xylocain 20 mg mL^{-1}, Astra, Söder Tälje, Sweden) + 5000 IU heparin (Heparin Leo 5000 IU mL^{-1}; Lövens) in 0·9% saline was infused through the middle lobe artery. The

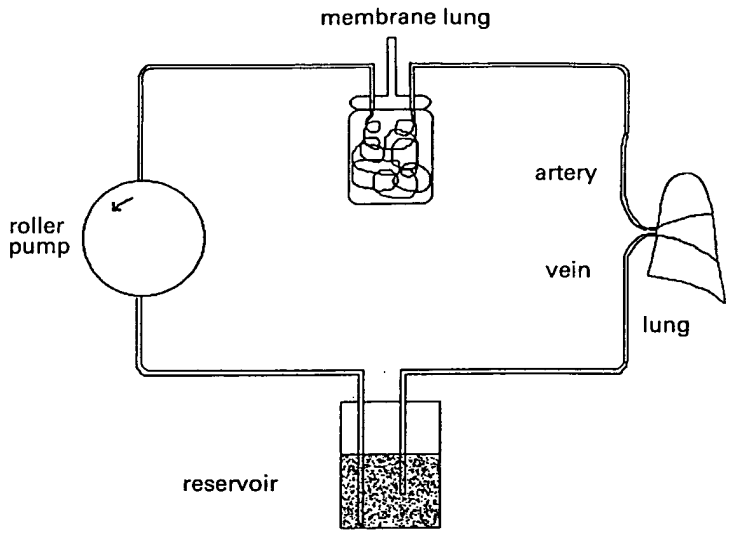

Figure 1 Schematic presentation of the closed-circuit organ perfusion system for the lung gene transfer.

adenoviral preparation (1×10^{11} pfu in 20 mL Krebbs-Ringer solution) was then infused into the arterial inlet, and the perfusion was immediately initiated. The flow rate was set at 100–120 mL min^{-1} initially and then adjusted to meet the acquired perfusion pressure by continuous straight pressure measurement in the arterial inlet. The perfusion pressure was kept at approximately 20/15 mmHg during the perfusion procedure. Viral perfusions were carried out for 60 min. Thereafter the cannules were removed and the puncture sites in the lobar artery and vein were sutured with 7–0 nonresorbable monofilament sutures (Prolene, Ethicon Inc., Somerville, New Jersey, USA). The circulation was restored immediately. The chest wall was closed with a suction chest tube *in situ*, which was removed during recovery from anesthesia. Hydrocortison (Solu-Cortef, Pharmacia & Upjohn, B-1130, Brussels, Belgium) 50 mg was administered intramuscularly after the operation. Buprenorphin (Temgesic, Reckitt & Colman, Great Britain) 5 $\mu g\ kg^{-1}$ was given intramuscularly every 8 h after the operation for analgesia during first day.

The animals were sacrified on the seventh postoperational day and the treated right middle lobe of the lung was removed for examination. Samples from the right middle lobe of the lung were prepared for histologic examination and expression of the *lacZ* gene was visually examined after staining with X-gal and immunohistochemical staining. Sample from the untreated left lung was prepared for analysis as a control.

Histochemical analysis and immunohistochemistry

Efficiency of adenoviral gene transfer was monitored by light microscopy of *lacZ* gene expression on cryosections. Ten samples of tissue were taken randomly from the perfused lung lobe and snap frozen in liquid nitrogen. Two sections were prepared from each sample for analysis. A total of 20 sections of treated lung tissue were examined per experiment. Cryostat sections of 5 μm thickness were first fixed for 10 min in 4% glutaraldehyde. Following extensive washings with 1 × PBS, the sections were incubated in a detergent solution containing 0·01% sodium deoxycholate, 0·02% NP40 and 2 mM magnesium chloride in PBS for 10 min. The sections were incubated in an X-gal solution (detergent solution containing 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 1 mg mL^{-1} X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside) for 3 h or overnight at 37 °C, and subsequently counterstained lightly with haematoxylin-eosin (HE). Possible cytotoxic and inflammatory changes were evaluated by histologic examination of formaldehyde-fixed and paraffin-embedded tissue sections after haematoxylin-eosin, periodic acid schiff, Perls' iron, May-Grünwald-Giemsa, van Gieson's and Verhoeff elastic stains.

Immunohistochemical detection of β-galactosidase was performed on cryosections. Tissue sections were first fixed in 4% paraformaldehyde. All washes were done with phosphate buffered saline (PBS). Samples were treated for 10 min with 3% H_2O_2 in methanol and for 2 h with 0·4% pepsin in 0·01 M HCl at 37 °C. Blocking of nonspecific binding was done by incubating for 20 min in room temperature with 1% bovine serum albumin in PBS followed by applying 1:50 to 1:100 dilution of rabbit polyclonal antiβ galactosidase (Rockland, Gilbertsville, Pennsylvania, USA) as a primary antibody. After overnight incubation at +4 °C, the peroxidase-conjugated goat antirabbit IgG (Jackson Immunoresearch laboratories Inc, USA) was used as a secondary antibody for one hour at room temperature (dilution 1:600). Peroxidase activity was revealed either by 3,3-diaminobenzidine tetrahydrochloride, DAB (Amresco, Dallas, Texas, USA) or 3-amino-9-ethylcarbazole, AEC (Zymed, San Francisco, USA). Sections were counterstained with haematoxylin.

The distribution of transgene expression was evaluated by light microscopy based on nuclear-dominant blue colour areas of X-gal staining or immunostaining for the β-galactosidase protein in the various cell types of the lung, i.e. alveolar epithelial cells, alveolar macrophages, capillary endothelial cells, as well as bronchial and bronchiolar epithelial cells. The quantification of expression was estimated by counting all uniformely stained bluish spots showing transgene expression in every 20 sections (area 100 mm^2 per section). The median number of spots per section and range of variation was calculated. Expression in the alveolar epithelial cells was evaluated visually on 10 high-power fields per section.

Results

The pilot experiment by *ex vivo* lung perfusion for 10 hours at 37 °C resulted in distinct transgene expression in both capillary endothelial cells and alveolar epithelial cells (data not shown).

The peroperative mortality of pigs was 27% in the *in vivo* perfusion experiments. Three animals died on the table because of arrhythmia at the beginning of the experiment. Only the surviving animals were included in the study. Gene transfer into lung by warm closed-circuit perfusion *in vivo* was performed succesfully for eight farm pigs. One perfusion was carried out for only 30 min and stopped earlier because of arrhythmia. However, this animal recovered from the operation normally. The other seven perfusion experiments were carried out for 60 min. The pH values in the perfusate during perfusion were between 7·40 and 7·43, and the oxygen saturation in the arterial part of the perfusate was 98·5–99%. After recovery from anaesthesia, the convalescence period of all the animals was uneventful.

Seven days after the perfusion *lacZ* transgene expression as judged by X-gal staining was found mostly in capillary endothelial cells and alveolar epithelial cells of both type I and type II pneumocytes (Fig. 2a,b). Some expression was also seen in bronchial and bronchiolar epithelial cells (Fig. 2c,d) as well as scattered alveolar macrophages. Some small arterioles showed expression in their endothelial cells (Fig. 2e). The immunohistochemical analysis confirmed the presence of β-galactosidase protein in both alveolar epithelial epithelial (type I and II pneumocytes) and alveolar endothelial cells (Fig. 3).

The distribution of transgene expression was relatively even throughout the specimens, except in the 30 min perfusion, which was completely negative. The quantitation of transgene expression was estimated by counting every nuclear-dominant blue area per section (Fig. 2f). For technical reasons, in places where the blue area consisted of numerous cells, it was counted as one uniformly stained single spot. Including all the seven gene transfer experiments that succeeded, the mean count of positive spots was 100 transgene expressing spots per 100 mm^2 section. The count varied between 3 and 365 spots per section. One reason for the seemingly inhomogenous distribution of the transgene was the size-matched sections – in some there was a large part of airway included and thus the total amount of cells was smaller than in sections including only alveolar tissue. Detailed results of each experiment are shown in Table 1. Expression was estimated to reach 2–5% of epithelial cells per perfused lobe.

In two experiments the untreated left control lung showed low intensity transgene expression in some alveolar macrophages (data not shown), but this putative endogenous activity was not seen in the other cells of the control lung tissue.

The macroscopic appearance of the lung lobe was slightly dark compared to other lobes seven days after the operation. The reason for this is not clear. However, there was no clear evidence for a venous outlet obstruction. The possible toxic and cytopathic effects were examined in parraffine embedded sections after haematoxylin-eosin, periodic acid schiff and some other special stainings (see Materials and methods). The special stainings showed only minor chronic inflammatory infiltrations and intra-alveolar edema in most of the cases, and alveolar hemorrage in one case. However, neither signs of diffuse alveolar damage nor hypertrophy of pulmonary muscular arterioles excluding pulmonary hypertension during perfusion were observed. No specific signs of reperfusion damage was found. The results suggest that the described gene transfer procedure is safe and does not cause any notable harmful lung tissue reactions. Some minor chronic inflammatory cell clusters containing mainly lymphocytes were remarkably less than we found previously in the kidney after the kidney perfusion experiments [27].

Discussion

The present study demonstrated the transfer of blood-borne adenovirus to about 2–5% of lung alveolar epithelial cells following one-hour closed-circuit perfusion of a lung lobe with a reporter gene containing adenovirus. The results may have significance for future gene therapy of lung disorders.

The lung is an important target for gene therapy, because this organ is often severely affected by both inherited and acquired diseases, and pulmonary complications of many diseases are often fatal. The lung is accessible for gene therapy by two routes, the airway or through the vascular system. Recombinant adenoviral vectors have been the most widely employed vehicles for gene delivery to the lung because of their natural tropism for the respiratory tract, and in comparison with cationic liposomes the overall transduction efficiency of the adenoviral vectors has been more efficient.

Most gene transfer studies into lung gene therapy and clinical trials have involved transfer through the airways. Lemarchand *et al.* [28] have shown the possibility of pulmonary gene transfer in sheep via the pulmonary artery

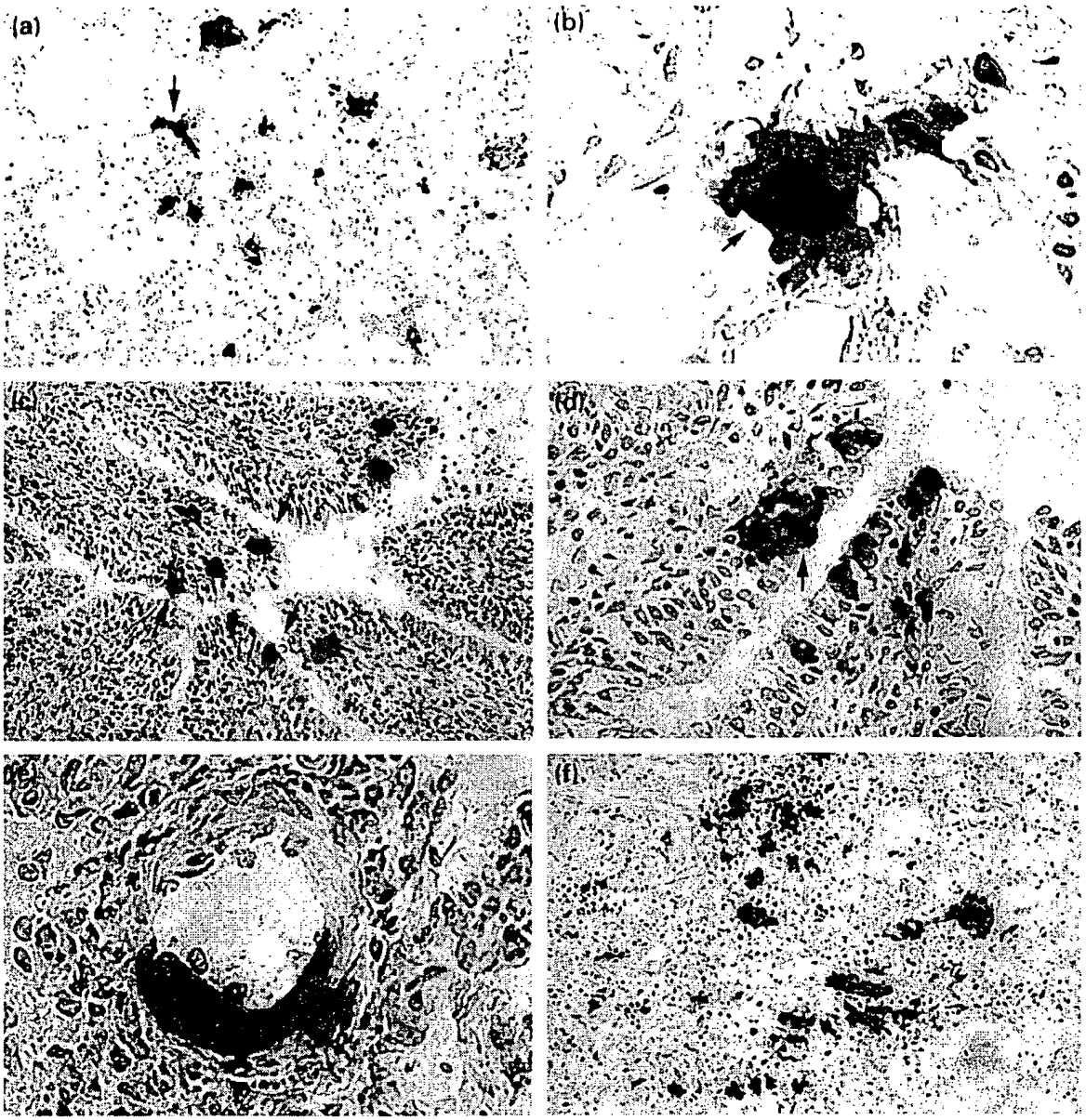

Figure 2 β-galactosidase gene expression in the lung tissue after 60 min closed-circuit perfusion examined after 7 days, X-gal staining. (a) type I pneumocyte (arrow), magnification ×205. (b) type II pneumocyte (arrow), magnification ×410. (c) and (d) β-galactosidase expression in the bronchial epithelial cells shown by arrows, (c) magnification ×102, (d) magnification ×205. (e) Expression in the wall of a small arteriole, magnification ×410. (f) The estimatated quantitity of expression by counting the clearly nuclear-dominant blue spots, here a total of 17 spots included, magnification ×102.

by a single infusion or by transient occlusion of the pulmonary artery and vein, which both provide quite a short interaction time with the vectors and pulmonary cells. They found some patchy exogenous gene expression in various categories of endothelial and also found patchy, mostly nonvascular cell transduction after intravascular infusions of adenoviral vectors into rodent lungs [19]. The present results – using adenovirus-mediated gene transfer into the porcine lung by prolonged incubation time for up to 60 min using a continuous closed-circuit warm organ perfusion method via vascular route – appeared to be more efficient than previous attempts. Thus, compared to the previously reported studies, our results with seven animals revealed relatively even gene transfer via vascular route into both airway and alveolar epithelial cells. The efficacy was, however, not calculated to exceed 5% of the epithelial cells, which may partly be explained by the barriers that the vector has to pass; first the capillary endothelial cell

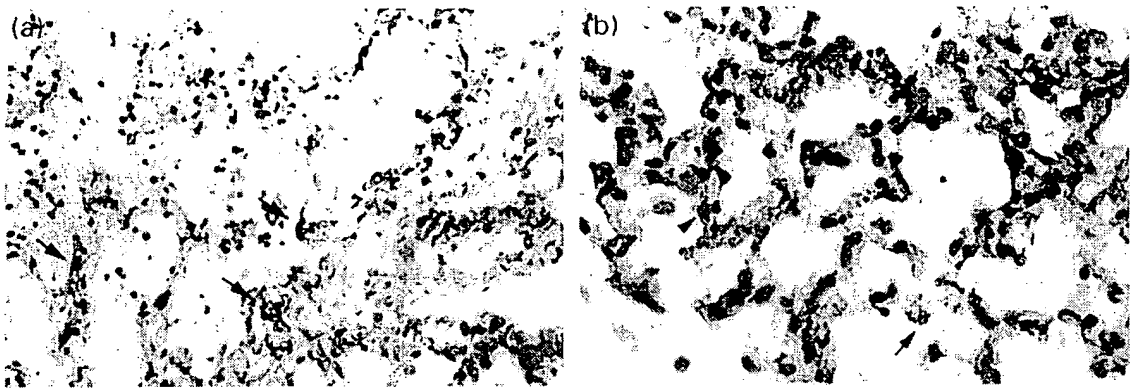

Figure 3 (a) β-galactosidase protein in the alveolar epithelial cells, type I pneumocytes (arrows), immunohistochemical staining, AEC, magnification ×205. (b) Expression in a spindle-shaped alveolar endothelial cell (arrowhead) and in a cuboidal cell on the alveolar wall, type II pneumocyte (arrow), immunohistochemical staining, AEC, magnification ×410.

and then the basement membrane to reach the epithelial cell in the alveoli.

We used oxygenation of the perfusate to hinder any ischemic alterations in the lung tissue during the perfusion, because for technical reasons the animal was intubated into the contralateral main bronchus during the perfusion and the treated lung was not inflated. Theoretically, by this method the incubation time of the vectors in the target tissue could be prolonged as long as necessary for adequate gene transduction to occur.

We have previously demonstrated the suitability of this perfusion method for targeted gene transfer into kidney glomeruli [25]. In the lung, gene transfer by perfusion is highly organ specific, but it does not seem to be very cell type specific. It is of significance, however, that the cells essential for expression, e.g. CFTR or surfactant protein B, i.e. bronchial and alveolar epithelial cells showed transgene expression. The requirements of gene transfer efficacy to correct the pulmonary manifestations of CF are not known exactly, but it has been estimated that even 6–10% expression in the epithelial cells may be sufficient for treatment of this disease [29]. An advantage with the vascular administration of vectors compared to aerosol delivery, especially in advanced CF is that the infected bronchiolar mucus layer may impair access of vectors to the cell surface via airways.

The prerequisite for successful gene transfer by closed-circuit perfusion is that the organ has a suitable blood circulation system. There should be at least one end artery and a suitable vein to collect the perfusate back into circulation. Organs such as kidney, lung, spleen and possibly liver are suitable target organs for this kind of gene transfer. During the perfusion the stable perfusion pressure is essential to prevent any pressure damage in the organ. So far, this closed-circuit perfusion method for gene transfer needs a major surgical operation. The next step will be to develop less invasive methods for perfusion, for example using catheterizations and possibly endoscopic methods in the future. The most obvious benefits of the *in vivo* perfusion method are organ specific gene transfer and the possibility of using small amounts of potentially expensive vector materials. Furthermore, the extracorporeal perfusion system diminishes the risk of administering large amounts of foreign genetic material to an immunocompetent individual, as the excess transgene material can be washed off from the organ following the organ perfusion.

The present results are particularly promising from the point of view of gene therapy for different lung diseases. Cystic fibrosis is an autosomal recessive disease affecting about 1 in 3000 Caucasian births. The major cause of morbidity and mortality in CF is pulmonary disease characterized by viscous mucus secretion, chronic bacterial infection, airway inflammation and premature death at around 29 years of age. Identification of the CFTR gene has led to vivid lung-directed gene therapy research targeting into gene therapy of CF [29]. Current understanding of the biology of cystic fibrosis lung disease suggests that vectors should express the transgene in mature, ciliated airway epithelia. Zabner *et al.* [24] found that the ability of adenovirus vectors to express a reporter gene and to correct defective cyclic AMP-stimulated Cl-transport in epithelial cells of CF patients in culture was

Table 1 Estimated efficiency of transgene expression by counting every blue coloured spot in specimens after X-gal and PAS staining. Twenty 100 mm^2 sections examined per perfused lung lobe after seven days

	Perfusion time (minutes)	Mean count of the transgene expressing areas (100 mm^2)	Range of variation
I	60	153	22–296
II	60	114	12–300
III	60	67	9–207
IV	30	–	–
V	60	91	9–217
VI	60	67	9–214
VII	60	185	104–365
VIII	60	22	3–72

Rosenthal ER, Dalemans W *et al.* In vivo transfer of the human cystic fibrosis transmembrane conductance regulator gene to the airway epithelium. *Cell* 1992;68:143–55.
5 Rosenfeld MA, Siegfried W, Yoshimura K, Yoneyama K, Fukayama M, Stier LE *et al.* Adenovirus-mediated transfer of a recombinant alpha 1-antitrypsin gene to the lung epithelium in vivo. *Science* 1991;252:431–4.
6 Lemarchand P, Jaffe A, Danel C, Cid MC, Kleinman HK, Stratford-Perricaudet LD *et al.* Adenovirus-mediated transfer of a recombinant human α1-antitrypsin cDNA to human endothelial cells. *Proc Natl Acad Sci USA* 1992;89:6482–6.
7 Li XM, Chopra RK, Chou TY, Schofield BH, Wills-Karp M, Huang SK. Mucosal IFN-gamma gene transfer inhibits pulmonary allergic responses in mice. *J Immunol* 1996;157:3216–9.
8 Dubinett SM, Miller PW, Sharma S, Batra RK. Gene therapy for lung cancer. *Hematol Oncol Clin N Am* 1998;12:569–94.
9 Yei S, Bachurski CJ, Weaver TE, Wert SE, Trapnell BC, Whitsett JA. Adenoviral-mediated gene transfer of human surfactant protein B to respiratory epithelial cells. *Am J Respir Cell Mol* 1994;11:329–36.
10 Boehler AD, Chamberlain Z, Xing AS, Slutsky M, Jordana J, Gauldie M *et al.* Adenovirus-mediated interleukin-10 gene transfer inhibits post-transplant fibrous airway obliteration in an animal model of bronchiolitis obliterans. *Hum Gene Ther* 1998;9:541–51.
11 Katkin JP, Gilbert BE, Langston C, French K, Beaudet AL. Aerosol delivery of a β-galactosidase adenoviral vector to the lungs of rodents. *Hum Gene Ther* 1995;6:985–95.
12 Yonemitsu Y, Kaneda Y, Muraishi A, Yoshizumi T, Sugimachi K, Sueishi K. HVJ (Sendai-virus)-cationic liposomes: a novel and potentially effective liposome-mediated technique for gene transfer to the airway epithelium. *Gene Ther* 1997;4:631–8.
13 Jeppsson A, Lee R, Pellegrini C, O'Brien T, Tazelaar HD, McGregor CG. Gene therapy in lung transplantation: effective gene transfer via the airways. *J Thorac Cardiovasc Surg* 1998;115:638–43.
14 Halbert CL, Standaert TA, Wilson CB, Miller AD. Successful readministration of adeno-associated virus vectors to the mouse lung requires transient immunosuppression during the initial exposure. *J Virol* 998;72:9795–805.
15 Pilewski JM, Engelhardt JF, Bavaria JE, Kaiser LR, Wilson JM, Albelda SM. Adenovirus-mediated gene transfer to human bronchial submucosal glands using xenografts. *Am J Physiol* 1995;268:L657–65.
16 Prince HM. Gene transfer: a review of methods and applications. *Pathology* 1998;30:335–47.
17 Wilson JM. Adenoviruses as gene-delivery vehicles. *N Engl J Med* 1996;334:1185–7.
18 Griesenbach U, Chonn A, Cassady R, Hannam V, Ackerley C, Post M *et al.* Comparison between intratracheal and intravenous administration of liposome-DNA complexes for cystic fibrosis lung gene therapy. *Gene Ther* 1998;5:181–8.
19 Schachtner SK, Rome JJ, Hoyt RF, Newman KD, Virmani R, Dichek DA. In vivo adenovirus-mediated gene transfer via the pulmonary artery of rats. *Circ Res* 1995;76:701–8.
20 Engelhardt JF, Yang Y, Stratford-Perricaudet LD, Allen ED, Kozarsky K, Perricaudet M *et al.* Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenoviruses. *Nat Genet* 1993;4:27–34.
21 Cusack JC, Spitz FR, Nguyen D, Zhang WW, Cristiano RJ, Roth JA. High levels of gene transduction in human lung tumors following intralesional injection of recombinant adenovirus. *Cancer Gene Ther* 1996;3:245–9.
22 Parsons DW, Grubb BR, Johnson LG, Boucher RC. Enhanced in vivo airway gene transfer via transient modification of host barrier properties with a surface-active agent. *Hum Gene Ther* 1998;9:2661–72.
23 Cassivi SD, Liu M, Boehler A, Tanswell AK, Slutsky AS, Keshavjee S. Transgene expression after adenovirus-mediated retransfection of rat lungs is increased and prolonged by transplant immunosuppression. *J Thorac Cardiovasc Surg* 1999;117:1–7.
24 Zabner J, Zeiher BG, Friedman E, Welsh MJ. Adenovirus-mediated gene transfer to ciliated airway epithelia requires prolonged incubation time. *J Virol* 1996;70:6994–7003.
25 Heikkilä P, Parpala T, Lukkarinen O, Weber M, Tryggvason K. Adenovirus-mediated gene transfer into kidney glomeruli using an ex vivo and in vivo kidney perfusion system – first steps towards gene therapy of Alport syndrome. *Gene Ther* 1996;3:21–7.
26 Engelhardt J, Yang Y, Stratford-Perricaudet LD, Allen ED, Kozarsky K, Perricaudet M *et al.* Direct gene transfer of human CFTR into human bronchial epithelia of xenografts with E1-deleted adenovirus. *Nature Genet* 1993;4:27–34.
27 Parpala-Spårman T, Lukkarinen O, Heikkilä P, Tryggvason K. A novel surgical organ perfusion method for effective ex vivo and in vivo gene transfer into renal glomerular cells. *Urol Res* 1999;27:97–102.
28 Lemarchand P, Jones M, Danel C, Yamada I, Mastrangeli A, Crystal RG. In vivo adenovirus-mediated gene transfer to lungs via pulmonary artery. *J Appl Phys* 1994;76:2840–5.
29 Alton E, Geddes D. The prospects for gene therapy in cystic fibrosis. *Curr Opin Pulm Med* 1995;1:471–7.
30 Schwaiblmair M, Vogelmeier C. Alpha 1-antitrypsin. Hope on the horizon for emphysema sufferers? *Drugs Aging* 1998;12:429–40.
31 Curiel DT, Pilewski JM, Albelda SM. Gene therapy approaches for inherited and acquired lung diseases. *Am J Resp Cell Mol* 1996;14:1–18.
32 Chapelier A, Danel C, Mazmanian M, Bacha EA, Sellak H, Gilbert MA *et al.* Gene therapy in lung transplantation: feasibility of ex vivo adenovirus-mediated gene transfer to the graft. *Hum Gene Ther* 1996;7:1837–45.
33 Van Ginkel FWC, Liu JW, Simecka JY, Dong T, Greenway RA, Frizzell H *et al.* Intratracheal gene delivery with adenoviral vector induces elevated systemic IgG and mucosal IgA antibodies to adenovirus and β-Galactosidase. *Hum Gene Ther* 1995;6:895–903.
34 Otake K, Ennist DL, Harrod K, Trapnell BC. Nonspecific inflammation inhibits adenovirus-mediated pulmonary gene transfer and expression independent of specific acquired immune responses. *Hum Gene Ther* 1998;9:2207–22.
35 Zsengeller ZK, Wert SE, Hull WM, Hu X, Yei S, Trapnell BC *et al.* Persistence of replication-deficient adenovirus-mediated gene transfer in lungs of immune-deficient (nu/nu) mice. *Hum Gene Ther* 1995;6:457–67.

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